REMARKS
ON
HAFFKINE'S METHOD OF PROTECTIVE INOCULATION AGAINST CHOLERA.

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As I have recently been subjected by M. Haffkine to his anti-cholera inoculations,[1] I propose to give an account of my experiences and a description of the method employed in the production of the vaccine.

In the course of his researches on the cholera vibrio which have been carried on during the last two years, M. Haffkine has met with many facts which tend to remove the difficulties that have hitherto stood in the way of accepting this microbe as the cause of cholera. Among these difficulties may be mentioned the impossibility of reproducing a disease in any way resembling human cholera by injections of Koch's vibrio into animals. M. Haffkine has recently succeeded in doing this in rabbits by a very simple and ingenious method. It is well known that the blood serum of the rabbit has the power of killing the cholera microbe. By cultivating the vibrio, first in diluted and then in undiluted rabbit's serum, M. Haffkine has succeeded in "acclimatising" it, so that at length it is completely unharmed by the bactericidal action in question. If a small quantity of a culture in rabbit's serum of these acclimatised vibrios be injected into the veins of a rabbit, the animal will succumb, showing symptoms which M. Haffkine assures me are extremely similar to those of typical cholera. During the collapsed condition that precedes death, cramps are frequently observed, and on *post-mortem* examination the intestine is found to be filled with the typical "rice-water" secretion. The latter, and also the mucous membrane of the intestine, contain the vibrios in large numbers. In some cases they have been found in the bile duct and ureter, but never in any other part of the body. Generally these symptoms appeared within a few days of inoculation, but more rarely the animal remained in apparently good health for periods extending to a couple of months before the symptoms developed. These latter experiments suggest an explanation of those anomalous cases in which a patient has been observed to succumb to a second attack of cholera during the same epidemic. Perhaps he had never been really free from the virus after his first attack. The cholera may have existed in a latent condition, as was the case with these rabbits. I owe my best thanks to M. Haffkine for his permission to mention these results. It is to be hoped that he will soon publish an account of them in detail.

In this, as in many other cases, the first step towards obtaining a protective vaccine was the preparation of an abnormally virulent form of the microbe, the so-called *virus fixe* or *virus exalté*. M. Haffkine succeeded in this by passing the microbe through a series of guinea-pigs. If a guinea-pig is inoculated into the peritoneum with a suspension of an agar culture of the microbe, it will succumb, and in the peritoneum is found an exudation containing a larger or smaller number of the vibrios. If this exudation is injected into the peritoneum of a second guinea-pig, the latter will also succumb, and so on with a third; but, unless certain precautions are taken, the series cannot be kept up. Each successive exudation will be found to contain fewer and fewer microbes, and at last the total number of microbes present will not be sufficient to kill the next guinea-pig in the series. To avoid this result a procedure has been adopted based on the following facts: The quantity of exudation formed varies in different cases. Usually, if a large guinea-pig is employed, a copious exudation, containing relatively few microbes, is produced. A small guinea-pig, on the other hand, yields a small quantity of exudation rich in microbes. Apparently the quantity of the exudation is a measure of the power of resistance possessed by the individual. M. Haffkine has based, on these facts, his method of passing the microbe through a series of animals. A guinea-pig is inoculated into the peritoneal cavity with cholera vibrios from an agar culture. On its death the peritoneal exudation is removed with a pipette, placed in a test tube, and left for several hours at the temperature of the room. If it is abundant (several cubic centimetres), it is then injected into the peritoneum of a small guinea-pig. If, on the other hand, it is small in quantity (1 cubic centimetre or less), it is injected into a fully-grown animal. On the death of the animals in the former case, the liquid injected will be found to have become concentrated; in the latter case, an increase in quantity will be observed. By attending to these precautions, the cholera virus can be passed through an indefinitely long series of animals. From each exudation in a series, an agar culture is made for the purpose of controlling the purity. Each successive culture will be found to be more virulent than its predecessors; also the guinea-pigs in the series die in shorter and shorter times after injection of the exudation. Between the twentieth and thirtieth passage the maximum degree of virulence appears to be reached. At this stage the peritoneal exudation is fatal to guinea-pigs in six to eight hours after its injection into the peritoneum. Agar cultures made from such an exudation also possess and retain this increased degree of virulence. Microbes from these cultures can kill rabbits and pigeons in doses which would be perfectly harmless if given in cultures of the ordinary degree of virulence. In fact, this *virus exalté* appears to be about twenty times as virulent as the ordinary form of the microbe.

As above stated, this *virus exalté* is rapidly fatal to guinea-pigs when injected into the peritoneum. It also kills them with certainty when introduced into the intestine or when given *per os* after neutralisation of the gastric juice with soda and quieting the intestines with opium according to Koch's method. It also differs from the ordinary form of the microbe in that it kills guinea-pigs when injected in small doses into the depth of the muscular tissue of the thigh.

If, however, it is injected under the skin, this *virus exalté* does not kill the animal; it produces a purely local malady. After a few hours an extensive œdema develops. This leads to necrosis of the tissues involved. In a few days the necrosed mass of tissue drops off, leaving a granulating wound. This at length heals completely. The animal is now found to be immune against inoculation with cholera in any way whatever, whether it is tested with ordinary or with strengthened virus, and whether it is introduced into the peritoneum, the intestine, or the muscular tissue. Obviously, however, such a method of inoculation could not be practically employed. It is necessary to devise some method of first protecting the guinea-pig against the necrosis produced by the *virus exalté*. This can be accomplished readily by a previous treatment with attenuated virus.

The attenuated virus is prepared by growing the microbe in a slow current of air. About 10 cubic centimetres of bouillon is placed in a flask having two lateral tubulures, and inoculated with the strengthened virus. The flask is placed in an incubator at a temperature of 39° C., and by means of a tube connected with one of the tubulures at one end, and, passing outside the incubator to a water-pump at the other, a slow current of air continually passes over the surface of the bouillon. The other tubulure of the flask is connected with a wash-bottle containing water and placed in the incubator, in order to saturate the entering air with aqueous vapour. This precaution is necessary to prevent undue evaporation, as the bouillon only forms a shallow layer at the bottom of the flask. Under these conditions the microbes produce a culture, but then rapidly die. It is therefore necessary to reinoculate them every third day into a fresh flask of bouillon. After each passage the microbes are found to be more and more attenuated. At last, after a series of passages under these conditions, the microbes are found to be so altered that they have completely lost the power of producing necrosis in guinea-pigs even when injected under the skin in exaggerated doses. They still, however, retain the power of

[1] Le Choléra Asiatique chez le Cobaye, *Comptes Rendus des Séances de la Société de Biologie*, July 9th, 1892. Le Choléra Asiatique chez le Lapin, et chez le Pigeon, *Ibid.*, July 19th, 1892. Inoculation de Vaccins Anti-cholériques à l'Homme, *Ibid.*, July 30th, 1892.

killing these animals when injected into the peritoneum in quantities only slightly larger than is necessary to attain this result with the ordinary virus.

If a dose of this attenuated virus (say one-eighth of a 24-hour old agar culture) is injected under the skin of a guinea-pig an extensive œdema develops within twenty-four hours. During the next few days this diminishes in size, leaving, however, a hard nodule, which persists for a considerable time. If a week later the animal is inoculated with a similar quantity of the strengthened virus an œdema develops, which is less than in the first case and does not lead to necrosis. When tested a few days later the animal is found to have acquired an immunity against the cholera microbe in whatever way it may be inoculated. This immunity has been found to be undiminished a couple of months after treatment.

Rabbits have also been made immune by the same method. They then show themselves refractory to every form of inoculation, including the method above described, which in the control animals leads to a disease resembling the cholera of human beings. M. Haffkine has also produced this immunity in pigeons. These facts led M. Haffkine to try the effect of these inoculations on himself, and afterwards on seven other gentlemen. The following is the account of my own case:

August 16th, midday. Dr. Roux inoculated me with one-eighth of a 24-hour old agar culture of the attenuated virus suspended in 1 cubic centimetre of bouillon in my left side, about 2 inches above the crest of the ilium. 1 P.M. Slight pain locally. 4 P.M. Swelling noticeable, and pain on movement. 8 P.M. Owing to pain on moving found walking about difficult. 10 P.M. Temperature began to rise; noticed a feeling of *malaise*. 12 midnight. Noticed temperature of 100°. This was the highest point reached.

August 17th. 7 A.M. Woke up; felt better; temperature normal. The swelling formed an area about 8 inches long and 2 or 3 wide, extending towards the inguinal lymphatic glands; painful on pressure, and reddened. 12 midday. During the day I worked as usual in the Institut Pasteur, but had a bad appetite and a feeling of fatigue, which once or twice impelled me to lie down for a short time. 3 P.M. The swelling extended during the day to the crest of the pubes and to within an inch of the umbilicus. This secondary extension of the swollen area was not painful on pressure, or reddened. 7 P.M. Felt bilious. 9 P.M. Took four capsules of castor oil.

August 18th. Woke up, feeling all right after a sound sleep; pain notably diminished; could walk about with ease, and went to a swimming bath.

August 19th. Swelling and redness of skin greatly decreased; pain on pressure trivial.

August 21st. 6 P.M. Inoculation with one-eighth of an agar culture of *virus exalté* suspended in bouillon. The injection was made on the centre of the inner side of the left arm. 11 P.M. Painful area extended to axilla; slight and occasional headache.

August 22nd. 4 A.M. Woke up; found it difficult to get out of bed owing to pain, but in a few minutes became accustomed to change of position, and walked about with ease; slight *malaise* and fever. 6 A.M. Felt better. 9 A.M. Breakfast, good appetite. During the day, August 22nd, slept most of the time, but was awakened every hour to take my temperature; in the evening felt bilious and constipated; took about ½ ounce of liquorice powder; the swelling now extended from the axilla nearly to the elbow; this area was very sharply defined by the redness of the skin. Slept well all night.

August 23rd. Woke up feeling quite well. Pain had decreased, but the swollen area had extended, reaching about 3 inches below elbow. This secondary extension of the swollen area was not painful on pressure, and the skin had a normal colour.

August 24th. Pain and swelling notably decreased. Skin beginning to assume a yellow hue.

August 25th. Further decrease of swelling. For an area measuring about 5 inches by 3, the skin was coloured yellow.

No enlarged glands were noticed all through the experiment. At present the only trace of the inoculation is a small hard nodule, less than half an inch in diameter, at each seat of injection.

With regard to these inoculations I should like to point out that although the œdematous swelling develops with startling rapidity, there is no reason for considering it as likely to lead to any dangerous inflammation. The cholera microbe is not one that is capable of producing pus. Knowing this fact, neither M. Haffkine or I made any attempt to keep quiet or rest ourselves after our inoculations. M. Haffkine happened to be busy after his first inoculation, and in spite of his fever worked for twenty-four hours continuously in the laboratory without tasting food. I worked as usual after my first inoculation. Two hours after my second inoculation I experienced the first part of my fever. It is noteworthy that both M. Haffkine and I had more fever after the second inoculation than after the first. All the other gentlemen who have been inoculated experienced only a trivial elevation of temperature and of discomfort after their second inoculation. Probably this is due to the fact that M. Haffkine and I had our second doses at comparatively short intervals after the first (five and six days respectively), and, as experiments on guinea-pigs indicate, these intervals were too short for the immunifying effect of the first dose to have completely developed.

The seven other cases presented symptoms essentially similar to my own, only, as above mentioned, the second inoculation (which was made after seven or eight days) produced far less general and local disturbance than the first. In two or three cases a transient constipation has been observed to follow the inoculation. In one case diarrhœa had been present for some days before the first inoculation, and vanished the day after it was performed.

M. Haffkine finds that it is also possible to vaccinate guinea-pigs by means of cultures previously sterilised by heat. In these cases the local reaction appears to be less than occurs after inoculation with living cultures. The immunity produced, however, does not seem to be of such a permanent character as that produced by the living microbe. It is possible, nevertheless, that for inoculations on human beings it may be well to commence with sterilised cultures, in order to diminish, as far as possible, the discomfort produced by later inoculations with the living virus.

I may mention here that in all his experiments M. Haffkine employs cultures on agar in preference to those in bouillon. The latter contain far more soluble poisons than the former, and there is reason for thinking that, as with the allied microbe the vibrio Metschnikovi, it is the less readily soluble substances contained in the bodies of the microbes that are concerned in the production of immunity.

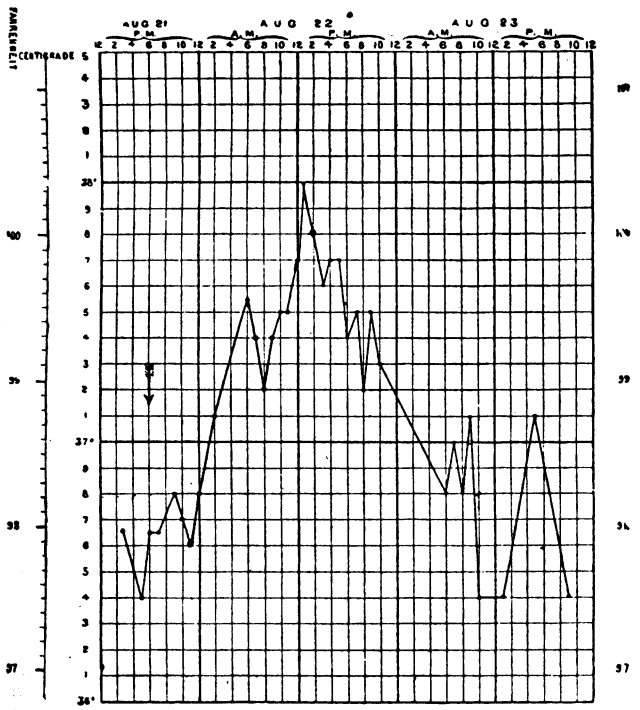
What proof have we that these inoculations are of any value whatever in protecting human beings against cholera? If the cholera vibrio is really the cause of this disease, the conditions of infection that obtain in Nature and in the laboratory must be widely different. How, then, can success in producing immunity against "laboratory infection" of a guinea-pig justify us in concluding that we have obtained immunity against "natural infection" of a human being. Obviously we have no direct and absolute demonstration of the value of these inoculations, and no doubt those people who need such proof before they are convinced will feel themselves justified in being "dead certain" of the futility of M. Haffkine's method for a considerable time to come.

In the first place it may be noted that the fact that there is a difference—it may be a great difference—between artificial and natural infection, though suggesting a source of error, is not of itself a reason for thinking that immunity against the one is not immunity against the other. In discussing the probability of an animal or a human being being immune against a particular microbe, we are concerned not so much with how the microbe can get into the system, but with what it does or does not do when it gets there. If any animal has been made immune against the cholera microbe, it has always been found to be capable of resisting it, in whatever way it may have been introduced into the body. Consequently, if a human being has been made immune against the effects of the cholera microbe when injected under the skin, analogy suggests that he is immune against the same microbe when it seeks to enter the system by the natural way, whatever that may be. But are those gentlemen who have been inoculated by M. Haffkine immune to the effects of the cholera microbe when inoculated under the skin? This is a matter which can be put to the test with great facility. The effects of a subcutaneous inoculation of the attenuated cholera microbe on a human being may be said now to be well known and easily recognised. There is a widespread and rapidly developing œdematous swelling and redness, which only vanish gradually after the lapse of several days. A small hard nodule also appears at the seat of inoculation. These changes may be regarded as a distinct character of the cholera inoculation. The fever and *malaise*, on the other hand, can not be so regarded; probably a similar injection of almost any microbe would produce a similar elevation of temperature.

Consequently, the presence or absence of a "local immunity" in a human being who has been subjected to M. Haffkine's treatment can be readily tested by a repetition of his first injection, namely, that of the attenuated virus. M. Haffkine has conceived the good idea of performing this experiment five weeks after his inoculation with the strengthened virus. After the injection a swelling rapidly developed, and as rapidly disappeared. It had almost gone in twenty-four hours, leaving no hard nodule, as had been the case with the previous

inoculations. This result is sufficiently striking when it is remembered that the œdema produced by the first inoculation in M. Haffkine's case took nine days to disappear. As might have been expected, a passing rise of temperature was produced, but this also took place in a guinea-pig that had received the same repeated inoculation, and that had previously been proved to be perfectly immune against cholera on several occasions.

Another objection suggests itself. Dr. Cunningham has isolated several distinct and permanent varieties (or, as he prefers to call them, species) of the cholera microbe. Will immunity against one of them confer immunity against another? M. Haffkine's vaccines have been prepared from a cholera microbe of Indian origin, and his inoculated guinea-pigs have shown themselves to be immune against this race. Considering the number of different varieties—a dozen or more—that Dr. Cunningham has described, it is, to say the least, improbable that a cholera microbe isolated two months ago from a case in the Necker Hospital in Paris should be of identically the same race as the one of Indian origin that has been so long in use in the Pasteur Institute. M. Haffkine finds that a guinea-pig that has been prepared by his method is just as refractory to one microbe as to the other.



Temperature curve after second inoculation with Haffkine's anti-choleraic vaccine. The injection was made on August 21st at 6 P.M., as indicated by the arrow.

Is this method of inoculation likely to confer a permanent immunity? To this question no definite answer can be given as yet. Guinea-pigs inoculated by the same method as has been employed for human beings have been found after two months to have lost none of their immunity. *A priori*, it is far more probable that an inoculation with *virus exalté* should confer a lasting immunity than a slight attack of cholera. In human beings the immunity can always be tested from time to time by a subcutaneous inoculation. In the event of a lasting œdema being produced by this test inoculation, it can form the starting point for a repeated treatment. It may be noted that there is no reason for thinking that this method of inoculation could be any source of danger to other individuals. There is, on the other hand, every reason for believing that the microbes injected are rapidly destroyed in the body, most probably *in situ*.

Lastly, it is important to remember that very probably after the first inoculation, less probably after the second, there may be a temporary diminution in the power of resisting the entry of the cholera microbes. Most probably within three or four days of the injection the power of resisting the attack of the cholera microbes has returned to normal, and after that time will continue to increase. If this is so, these inoculations should not be practised in a given place at the time that an epidemic is raging, without great caution. Before forming a definite conclusion with regard to this, further experiments on animals are needed.

The evidence at present existing shows that M. Haffkine's method of inoculation is not attended by any grave disturbance of health, and that it can be practised on human beings with perfect safety. The fact that it produces immunity against cholera in any form, in animals of such widely different organisation as guinea-pigs and pigeons, gives reason for hoping that it may produce an equally good effect in human beings, but it must necessarily be a long time before we can possess any direct evidence of any value on this point.

SIXTIETH ANNUAL MEETING

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PROCEEDINGS OF THE SECTIONS.

PATHOLOGY.

A CASE OF APHASIA AND RIGHT HEMIPLEGIA,

WITH TEMPORARY SPASMODIC CONJUGATE DEVIATION OF THE EYES, EXCITED BY ATTEMPTS TO CONVERGE THE EYES STRONGLY TOWARDS THE MIDDLE LINE.

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I.—*Preliminary Remarks.*

I AM indebted to Dr. W. Ewart, of St. George's Hospital, London, for permission to publish this case, and to use his clinical notes, and to Dr. Richard Sisley, who was at that time Registrar, and has also furnished me with additional notes of great interest. The clinical details, with the exception of those I observed myself, have been abstracted from the notes which these two gentlemen have so kindly placed at my disposal.

Early in May, 1889, Dr. Sisley was showing me some interesting cases in the wards, and we came to the paralysed patient whose case I am about to relate. The extent of the paralysis made me wonder how far forward the softening had extended in the frontal lobe. In a previous case of actinomycotic abscess of the brain,[1] I had noticed a connection between dilatation of the right pupil and affection limited to the hindmost part of the head area.

II.—*Special Observations.*

In this case the head area was certainly much implicated. There was no dilatation of the right pupil. There being no evidence of dilatation or contraction, I tried to see whether there would be any irregularity in the mode of contraction and dilatation of the pupil during accommodation. In order to stimulate the two eyes to the same extent, I moved my finger in the mesial plane from a distance of about three or four feet towards the root of the nose of the patient. The patient followed my finger with both eyes, nothing remarkable being observed till the finger got to about ten to six inches from the eyes, when suddenly the right eye, which till then had converged in the same way as the left, turned to the right, and its axis became parallel to that of the left. The movement was strikingly sudden. I was so taken by surprise that I forgot to observe the pupil. I repeated the observation two or three times, and the phenomenon recurred each time.

In addition to this, I noticed a remarkable degree of œdema of the right hand. This was so marked when the two hands

[1] *Trans. Path. Soc.*, 1889.